

# THE ONHED SHATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME;

ADSH Research Joundation

ALCCCAS, THERE HAS BEEN PRESENTED TO THE

## Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED DISTINCT VARIETY OF SEXUALLY REPRODUCED, OR TUBER PROPAGATED PLANT, THE MAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF TWENTY GARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC LIPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE VIBEL OF EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR PRING IT, OR EXPORTING IT, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE BURPOSES, OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT BY THE PLANT VARIETY PROTECTION ACT. (84 STAT. 1542, AS AMENDED, 7 U.S.C. 2321 ET SEQ.)

OAT

'Souris'

In Jestimonn Marrest, I have hereunto set my hand and caused the seal of the Hant Harista Protection Office to be affixed at the City of Washington, D.C. this sixteenth day of Way, in the year two thousand and eight.

Allado

Benzu

Commissioner
Plant Variety Protection Office
Agricultural Marketing Service

Solmand T. Schafe

riculture

(See reverse for instructions and information collection burden statement)

GENERAL INSTRUCTIONS: To be effectively filed with the Plant Variety Protection Office (PVPO), ALL of the following items must be received in the PVPO: (1) Completed application form signed by the owner; (2) completed exhibits A, B, C, E, F; (3) for a tuber reproduced variety, verification that a viable (in the sense that it will reproduce an entire plant) tissue culture will be deposited and maintained in an approved public repository; and (4) payment by credit card or check drawn on a U.S. bank for \$4,382 (\$518 filing fee and \$3,864 examination fee), payable to "Treasurer of the United States" (See Section 97.6 of the Regulations and Rules of Practice). NEW: With the application for a seed reproduced variety or by direct deposit soon after filling, the applicant must provide at least 3,000 viable untreated seeds of the variety per se, and for a hybrid variety at least 3,000 untreated seeds of each line necessary to reproduce the variety. Partial applications will be held in the PVPO for not more than 90 days; then returned to the applicant as un-filed. Mail application and other requirements to Plant Variety Protection Office, AMS, USDA, Room 401, NAL Building, 10301 Baltimore Avenue, Beltsville, MD 20705-2351. Retain one copy for your files. All items on the face of the application are self explanatory unless noted below. Corrections on the application form and exhibits must be initialed and dated. DO NOT use masking materials to make corrections. If a certificate is allowed, you will be requested to send a payment by credit card or check payable to "Treasurer of the United States" in the amount of \$768 for issuance of the certificate. Certificates will be issued to owner, not licensee or agent.

**NOTES:** It is the responsibility of the applicant/owner to keep the PVPO informed of any changes of address or change of ownership or assignment or owner's representative during the life of the application/certificate. The fees for filing a change of address; owner's representative; ownership or assignment; or any modification of owner's name is specified in Section 97.175 of the regulations. (See Section 101 of the Act, and Sections 97.130, 97.131, 97.175(h) of the Regulations and Rules of Practice.)

**Plant Variety Protection Office** 

Telephone: (301) 504-5518

FAX: (301) 504-5291

General E-mail: PVPOmail@usda.gov

Homepage: http://www.ams.usda.gov/science/pvpo/PVPindex.htm

#### **SPECIFIC INSTRUCTIONS:**

To avoid conflict with other variety names in use, the applicant must check the appropriate recognized authority and **provide evidence** that the permanent name of the application variety (even if it is a parental, inbred line) has been cleared by the appropriate recognized authority before the Certificate of Protection is issued. For example, for agricultural and vegetable crops, contact: U.S. Department of Agriculture, Agricultural Marketing Service, Livestock and Seed Programs, **Seed Regulatory and Testing Branch**, 801 Summit Crossing Place, Suite C, Gastonia, North Carolina 28054-2193 Telephone: (704) 810-8870. http://www.ams.usda.gov/lsg/seed.htm.

#### ITEM

19a. Give:

- (1) the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method;
- (2) the details of subsequent stages of selection and multiplication;
- (3) evidence of uniformity and stability; and
- (4) the type and frequency of variants during reproduction and multiplication and state how these variants may be identified
- 19b. Give a summary of the variety's distinctness. Clearly state how this application variety may be distinguished from all other varieties in the same crop. If the new variety is most similar to one variety or a group of related varieties:
  - (1) identify these varieties and state all differences objectively;
  - (2) attach replicated statistical data for characters expressed numerically and demonstrate that these are clear differences; and
  - (3) submit, if helpful, seed and plant specimens or photographs (prints) of seed and plant comparisons which clearly indicate distinctness.
- 19c. Exhibit C forms are available from the PVPO Office for most crops; specify crop kind. Fill in Exhibit C (Objective Description of Variety) form as completely as possible to describe your variety.
- 19d. Optional additional characteristics and/or photographs. Describe any additional characteristics that cannot be accurately conveyed in Exhibit C. Use comparative varieties as is necessary to reveal more accurately the characteristics that are difficult to describe, such as plant habit, plant color, disease resistance. etc.
- 19e. Section 52(5) of the Act requires applicants to furnish a statement of the basis of the applicant's ownership. An Exhibit E form is available from the PVPO.
- 20. If "Yes" is specified (seed of this variety be sold by variety name only, as a class of certified seed), the applicant MAY NOT reverse this affirmative decision after the variety has been sold and so labeled, the decision published, or the certificate issued. However, if "No" has been specified, the applicant may change the choice. (See Regulations and Rules of Practice, Section 97.103).
- 23. See Sections 41, 42, and 43 of the Act and Section 97.5 of the regulations for eligibility requirements.
- 24. See Section 55 of the Act for instructions on claiming the benefit of an earlier filing date.
- 22. CONTINUED FROM FRONT (Please provide a statement as to the limitation and sequence of generations that may be certified.)
- 23. CONTINUED FROM FRONT (Please provide the date of first sale, disposition, transfer, or use for each country and the circumstances, if the variety (including any harvested material) or a hybrid produced from this variety has been sold, disposed of, transferred, or used in the U.S. or other countries.)

#### See attached page. Identified as #23.

24. CONTINUED FROM FRONT (Please give the country, date of filing or issuance, and assigned reference number, if the variety or any component of the variety is protected by intellectual property right (Plant Breeder's Right or Patent).)

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0581-0055. The time required to complete this information collection is estimated to average 1.4 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

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#200800063

# PVP Application for 'Souris' oat (ND961161) by NDSU Research Foundation - CONTINUED

**#23.** 'Souris' was first evaluated under a Material Transfer Agreement (MTA) in Canada, April 18, 2006. MTA's were used since, as well, and were for testing and evaluation only. No seed sales were allowed. 'Souris' was licensed in Canada in March 2007, and Plant Breeders' Rights in Canada were applied for on April 9, 2007. 'Souris' was evaluated in Canada in 2007, under the license. 'Souris' was distributed to the North Dakota Crop Improvement Association under contract for seed increase in April 2007.

# **PVP Application for 'Souris'**

# Question 24.

A variety named 'Dal" is in the early/distant parentage of Souris and was PVP protected. PVP was issued in 1979 as Certificate No. 7200136.

# 19 a. Exhibit A. Origin and Breeding History of 'Souris'

Pedigree

ND90141/ND900118

ND90141 = ND894898/ND852107 ND894898 = R801441/ND820712

R801441 = synthetic hexaploid derived from an *Avena* magna / A. longiglumis hybrid by P. Rothman. ND820712 =

M23/RL3038//Otana/3/Froker/RL3038//'Hudson' M23 = 'Avon'//'Rodney'/'Milford' RL3038 is a breeding line received from R. McKenzie (Agric. & Agri-Food Canada Res. Stn., Winnipeg, MB. RL3038 has a complex pedigree that includes 'Rodney' and 'Pendek' and possesses genes *Pc-38*, *Pc-39*, *Pg-2*, and *Pg-13*.

ND852107 = ND810603/'Otana' ND810603 = 'Kelsey/'Dal'/RL3038/Dal

ND900118 = MN78142/ND852158 MN78142 = 'Otter'/3/'Garland'/PI267989//MN836/Avon

Experimental Designation ND961161

#### Exhibit A. Origin and Breeding History of 'Souris' 19 a.

Breeding Method –

Modified single seed descent and pedigree method

Selection and Multiplication –	Stage of development	Selection Criteria
1992 Fall greenhouse	Final cross	
1993 Spring greenhouse	F <sub>1</sub>	F <sub>1</sub> plants were uniform and seed from 5 plants was bulked to produce F <sub>2</sub> population
1993 Field	F <sub>2</sub> selection of single panicle	F <sub>2</sub> population was segregating for crown rust and stem rust resistance in the field. Plants resistant to both crown rust and stem rust were selected for advancement.
1993 Fall greenhouse	F <sub>3</sub> single seed descent accompanied by screening for seedling resistance to critical races of stem and crown rust.	Seedlings were inoculated with composite of crown rust races that were avirulent on Pc-91 and with stem rust race NA27. Seedlings exhibiting a resistant infection type were grown to maturity and seed from individual resistant F <sub>3</sub> plants were advanced to the field.
1994 Field	F <sub>4</sub> planted in hill plots from seed of single F <sub>3.4</sub> panicle F <sub>4</sub> panicles harvested from selected hill plots	Panicles from plants in hill plots exhibiting stem rust and crown rust resistance along with resistance to lodging and tolerance to barley yellow dwarf virus were harvested to provide seed for advancement to the F <sub>5</sub> .

# 19 a. Exhibit A. Origin and Breeding History of 'Souris'

Breeding Method – Modified single seed descent and pedigree method

Selection and Multiplication –	Stage of development	Selection Criteria
1995 Field	Seed from F <sub>4</sub> panicle planted to produce paired hill plots. Selected paired hill plot harvested to produce F <sub>4:5</sub> breeding line ND961161 that became the source of Souris breeder's seed.	Hill plots exhibiting homogeneity of crown rust resistance and stem rust resistance were selected for harvest. Lodging resistance, white hull color, and visual selection of kernel morphology were considered to further select plots that were identified for harvest. Harvested lines were evaluated as seedlings in the greenhouse using stem rust race NA27 and a composite of crown rust races to identify lines homogeneous for resistance to these diseases. These selected lines were
1996 Field	F <sub>6</sub> Preliminary screening trial – Unreplicated trial with repeating checks for purposes of comparison. 4-row plots.	advanced to the F <sub>6</sub> generation.  Selection was based on lodging resistance, medium heading date, high grain yield, high test weight, kernel morphology, and resistance to stem and crown rust in the field.  Stem rust and crown rust seedling resistance evaluation was repeated in the greenhouse.  The experimental designation ND961161 was assigned from this trial.

# 19 a. Exhibit A. Origin and Breeding History of 'Souris'

Breeding Method – Modified single seed descent and pedigree method

	Selection and Multiplication –	Stage of development	Selection Criteria
	1997 Field	F <sub>7</sub> Preliminary yield trial — two locations, two replications	Selection was based on lodging resistance, medium heading date, high grain yield, high test weight, high groat percentage, and resistance to stem and crown rust in the field.  Stem rust and crown rust seedling resistance evaluation was repeated in the greenhouse to identify homogeneous resistant lines.
7.00	1998 Field	F7 Advanced yield trial – Four locations, three replications per location	Selection was based on lodging resistance, medium heading date, high grain yield, high test weight, high groat percentage, and resistance to stem and crown rust in the field.  Stem rust and crown rust seedling resistance evaluation was repeated in the greenhouse.
- market	1999 Field	F <sub>8</sub> Tri-State Oat Nursery, 3 ND, 3 MN, and 3 SD locations Increase plot rougued of tall variants to initiate production of breeder seed	Evaluation was based on lodging resistance, medium heading date, high grain yield, high test weight, high groat percentage, and resistance to stem and crown rust in the field. Stem rust and crown rust seedling resistance was evaluated in the greenhouse.

#### 19 a. Exhibit A. Origin and Breeding History of 'Souris'

Breeding Method – Modified single seed descent and pedigree method

Selection and Multiplication –	Stage of development	Selection Criteria
2000 Field	F <sub>9</sub> North Dakota Oat Variety	ND961161 that became Souris
	Trials at ten locations	was determined to produce
	(NDOVT) and UMOPN at	high grain yield, medium high
	22 locations. Increase plot	test weight, and white hull
	evaluated for homogeneity	color. Stem rust and crown
	and tall variants were	rust resistance was evaluated at
	removed.	many locations and ND961161
•		was identified to have stable
		crown rust resistance and
		resistance to stem rust race NA27. Stem rust and crown
		rust seedling resistance
		evaluation was repeated in the
	·	greenhouse.
2001 Field	F <sub>9</sub> North Dakota Oat Variety	ND961161 that became Souris
	Trials at ten locations	was determined to produce
	(NDOVT) and UMOPN at	high grain yield, medium high
•	22 locations. Increase plot	test weight, and white hull
•	evaluated for homogeneity	color. Stem rust and crown
•	and tall variants were	rust resistance was evaluated at
	removed.	many locations and ND961161
		was identified to have stable
		crown rust resistance and
		resistance to stem rust race
		NA27. Stem rust and crown
		rust seedling resistance
		evaluation was repeated in the greenhouse.
		greennouse.

# 19 a. Exhibit A. Origin and Breeding History of 'Souris'

Breeding Method –

Modified single seed descent and pedigree method

Selection and Multiplication –	Stage of development	Selection Criteria
2002 ND Field	NDOVT at 10 locations and UMOPN	ND961161 that became Souris was determined to produce high grain yield, medium high test weight, high groat betaglucan concentration, and white hull color. Stem rust and crown rust resistance was evaluated at many locations and ND961161 was identified to have stable crown rust resistance to stem rust race NA27
2003 Field	F <sub>11</sub> NDOVT at 10 locations	Evaluation continued for all characteristics evaluated in 2002
2004 Field	F <sub>12</sub> NDOVT at 10 locations	Evaluation continued for all characteristics evaluated in 2003
2005 Field	F <sub>13</sub> NDOVT at 10 locations Preliminary increase by Foundation Seed Stocks Project	Evaluation continued for all characteristics evaluated in 2004
2006 Field	F <sub>14</sub> NDOVT at 10 locations Distribution of Foundation Seed and release as cultivar	Evaluation continued for all characteristics evaluated in 2005

# Evidence of uniformity and stability:

Souris has been observed to be uniform and stable for stem rust resistance and crown rust resistance for ten generations from the original  $F_{4:5}$  that was designated ND961161 in 1996 until release in 2006. Souris appears otherwise uniform and stable.

The type and frequency of variants during reproduction and multiplication and how these variants may be identified:

Lemma and palea are white and 93% of lemmas are fluorescent under irradiation with a UV light source while approximately 7% are weakly fluorescent or non-fluorescent.

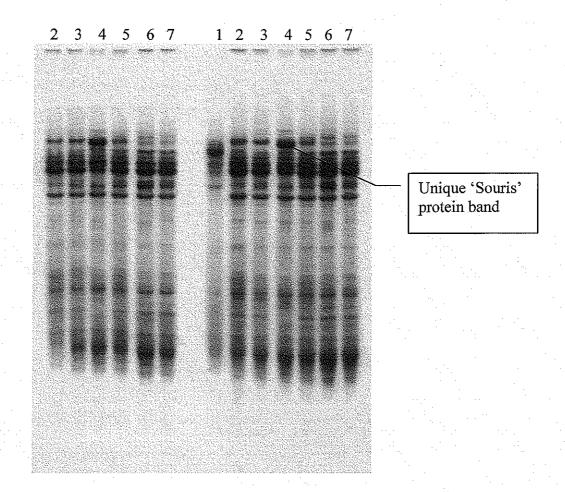
Awns are normally absent, but weak awns may occur under some environmental conditions. A few tall variants (0.5%) may be present under some conditions. These naturally occurring tall variants are obvious under some environmental conditions that favor increased plant height.

19 b. Exhibit B. Statement of distinctness.

'Souris' is a spring oat that is most similar to 'HiFi' in appearance. Souris, like HiFi, possesses a crown rust resistance gene, Pc-91, derived from Amagalon, a synthetic hexaploid developed from a cross between Avena longiglumis and A. magna developed by P.G. Rothman. Evidence for the presence of Pc-91 is provided by the highly resistant (;) seedling infection type after inoculation with a composite (NDCRC05) of isolates collected in North Dakota during the past 10 years (Exhibit D, Table 4). This reaction distinguishes Souris and HiFi from other North American oat cultivars. Souris also possesses resistance to stem rust race NA27 conferred by Pg-13 plus other unknown resistance genes as indicated by seedling infection type 1 (moderately resistant) when inoculated with stem rust race NA27 (Exhibit D, Table 5). Souris and HiFi are similar in appearance to 'Otana', but can be readily distinguished from Otana since Otana is susceptible to the critical races of stem rust and crown rust while Souris and HiFi produce resistant reactions when challenged with these races (Exhibit D, Tables 4 and 5). Souris can be distinguished from HiFi by seed protein polyacrylamide gel electrophoresis (PAGE) as illustrated in the attached figure. The PAGE procedure for oats was modified by the North Dakota State Seed Department (NDSSD) from the ISTA (International Seed Testing Association) publication procedure for variety testing of wheat. The NDSSD modified procedure uses a modification of the gel run time. The ISTA procedure is referenced in the 1992 Handbook of Variety Testing edited by R.J. Cooke. The procedure is on pages 2-5 through 2-6. A copy of the relevant section of the handbook (pages 2-5 and 2-6) is attached. The modified procedure for oat seed protein analysis used by the NDSSD is also attached. Application of the PAGE procedure to Souris, HiFi, and Morton provides a clear distinction in protein banding pattern to distinguish Souris from the other cultivars.

8/21/07

# Oat Seed Protein Electrophoresis Test Results



NDSSD Acid PAGE Analysis of Oat seed protein. Samples submitted for testing on 8-09-07 by NDSU Plant Science Department (Dr. McMullen). Sample lanes represent: 1 = Morton Oat control; 2 = HiFi Oat control; 3 = L2700212 (HiFi sample); 4 = L2700211 (Souris sample); 5 = L2700213 (ND030291 sample); 6=L2700214 (ND030288 sample); 7=L2700215 (ND030299 sample). Source: Jeff Prischmann, Diagnostic Lab Manager, NDSSD.

#### A. 1. Principle

The alcohol-soluble proteins (gliadins from wheat, hordeins from barley) are extracted from seeds and separated by PAGE at pH 3.2. The pattern of protein bands produced (electrophoregram) is related to genetic constitution and can be considered as a 'fingerprint' of a variety. The 'fingerprints' can be used to identify unknown samples and mixtures, by single seed analysis.

As a guideline, it is recommended that 100 seeds are used. Very precise estimates of varietal purity may require a larger sample. If a comparison is being made with a standard value, sequential testing using batches of 50 seeds can be undertaken in order to minimise the workload. A simple check on the identity of a single major constituent of a seed lot can be done using less than 50 seeds.

#### A. 2. Apparatus and Equipment

A. 2.1 The Pharmacia GE-2/4 electrophoresis apparatus and EPS 400/500 power supply have been successfully used, but any suitable vertical electrophoresis system eg. Desaga, BioRad, Biometra should give comparable results.

#### A. 2.2 Chemicals

All chemicals should be of 'Analytical Reagent' grade or better.

Acrylamide ('specially purified for electrophoresis')
Bisacrylamide ('specially purified for electrophoresis')

Glacial acetic acid

Glycine

Ferrous sulphate

Ascorbic acid

Hydrogen peroxide (or ammonium persulphate and TEMED)

Monothioglycerol (or 2-mercaptoethanol)

Pyronin G (or methyl green)

Trichloroacetic acid

Ethanol

2-chloroethanol

PAGE Blue G-90 (or PAGE Blue 83) (or any reagent equivalent to the 'Coomassie Brilliant Blue' G or R series of dyes).

#### A. 2.3 Solutions

#200800063

#### A. 2.3.1 Extraction solution

Wheat: Pyronin G (or methyl green)	0.05%
2-chloroethanol	25%

Keep cold.

Barley: Pyronin G (or methyl green)	0.05%
2-chloroethanol	20%
containing Urea	18%
monothioglycerol	1%
(or 2-mercaptoethanol	)1%

Keep cold or prepare fresh.

#### A. 2.3.2 Tank buffer solution:

for the second second	100		
Glacial acetic a	acid	4 ml	
Glycine		0.4 g	

Made up to 11 with water; keep cold.

#### A. 2.3.3 Gel buffer solution:

	the state of the state of	
Glacial acetic acid		20 ml
Glycine		1.0 g

Made up to 11 with water; keep cold.

#### A. 2.3.4 Staining solution:

1	Trichloroacetic acid Water	100 g
2	PAGE Blue G-9 (or PAGE Blue 83) Ethanol	1 g 100 ml

#### A. 3. Procedure

#### A. 3.1 Protein extraction

Single seeds are crushed with pliers or a similar implement and transferred to 1.5 ml polypropylene centrifuge tubes or to the wells of a micro-titer plate. Extraction solution (A. 2.3.1) (0.2 ml for wheat, 0.3 ml for barley) is added, the contents of the tubes or plates are thoroughly mixed and the tubes are allowed to stand (covered or sealed) overnight at room temperature. The tubes are centrifuged at 18000 xg and the supernatants used for electrophoresis.

#### A. 3.2 Preparation of the gel

Clean and dry gel cassettes are assembled, according to the design of the equipment. Treating the glass plates with silicon prior to assembly can facilitate subsequent removal of the gel. The gel cassettes can incorporate a plastic backing sheet (eg 'Gel Bond PAG', FMC Corporation). This supports the gel during subsequent operations.

#### Gel Medium

60 ml <i>ca</i>
10 g
0.4 g
6 g
0.1 g
0.005 g

Stir the solution and make up to 100 ml with

Stock gel buffer (A.2.3.3.)

Add, mixing quickly

freshly prepared 0.6% (v/v) Hydrogen peroxide 0.35 ml per 100 ml gel medium

Pour the gel.

(Note: the gel mixture can be cooled to near freezing prior to the addition of the peroxide.)

Polymerisation should be complete in 5–10 minutes. If not, it may be necessary to adjust the concentration of hydrogen peroxide added. An acrylic 'comb' is placed in the top of the cassette, to make wells in the gel. The gel mixture should over-fill the cassette, or be over-layed with water, to ensure satisfactory polymerisation of the upper surface.

Note that as an alternative to the hydrogen peroxide catalyst, it is possible to use ammonium persulphate (0.1 ml of 10% solution, freshly prepared) and TEMED (0.3 ml) added to the gel mixture prior to pouring the gel.

#### A. 3.3 Electrophoresis

The acrylic comb is removed from the gel and the sample wells washed with tank buffer (A.2.3.2). The tank is filled with an appropriate volume of buffer (A. 2.3.2) (depending on the equipment used). Samples (10–20 µl) are loaded into the wells and the gel placed in the tank, ensuring that the sample wells are completely filled. Electrophoresis is carried out at no more than 500 V (constant voltage) for twice the time taken for the pyronin G marker dye to leave the gel, or three times if methyl green is used as a tracking dye. It must be remembered that the anode (positive electrode) is at the origin (top of the gel) in this system and the polarity of the electric field should be adjusted accordingly. Water should be circulated through the buffer tank to maintain the temperature at 15–20° C.

#### A. 3.4 Fixing and staining

The gel cassette is removed from the tank, opened and the gel placed in a plastic or glass box containing 5–10 ml of 1% PAGE Blue G90 (or PAGE Blue 83) in 200 ml of 10% trichloroacetic acid (A.2.3.4). Staining is complete in 1–2 days at room temperature and de-staining is not usually needed. Precipitated stain should be scraped from the surface of the gel. The gel is washed in water to enhance the stain and can then be examined or photographed. Any blue background in the gel is removed by washing in 10% trichloroacetic acid. Gels can be stored in polythene bags at 4° C for many months without deterioration.

Typical results produced using the above procedure, and methods of utilising and reporting the electrophoretic data are presented in Section 3 of the Handbook.

One of the benefits of the ISTA standard reference method is that it can be utilised, with little or no modification, for prolamin analysis and variety identification in other cereals such as oats, durum wheat, triticale and rye (4). The Electrophoresis Working Group will be organising a collaborative test of the method for oats identification, with a view to including this in the International Rules. Rice and maize varieties have also been reported as being successfully analysed using essentially this procedure.

# ISTA Varietal Identification Procedure for Wheat and Oats Using Bulked Seed Analysis (North Dakota State Seed Department Protocolrevised 2006)

- Sample preparation: Place approximately 100 seed into a coffee grinder and grind for about 20 seconds. Pass sample through a #9 and #4 dodder/purity test sieves (Hoffman Manufacturing, Inc.). Weigh out 0.15g of ground and sieved sample and place into a 1.8ml capped centrifuge tube containing 0.6ml of 2CE extraction buffer (see recipe below). Vortex and incubate overnight a 4°C. Prior to use, centrifuge at 8000g for 5 minutes. Apply 6-8ul of supernatant to the gel. Prepare control samples in the same manner as the samples.
- 2. Gel preparation: Prepare a 10% acrylamide gel using the reagents and procedures listed below. The gel should be prepared a day in advance or 2 hours prior to usage. Glass plates are cleaned and coated with Rain-X to aid in gel removal from the glass. Our lab uses the Bio-Rad Protein II system with 16x20cm gels and a 25 well comb. Electrophoresis is run using a cooling system to maintain a gel temperature of 20°C.
- **3.** Electrophoresis: Electrophoresis is carried out under constant voltage of 500 volts for 5.5 hours for wheat and 2.5 hours for oats.
- 4. Gel staining: After electrophoresis, gels are removed and placed into trays containing 300 ml distilled water with 50 g trichloroacetic acid and 10ml of 2% coomassie blue G-250 in 95% EtOH (see below). Stain gels overnight for best results. Destain gels as necessary with water to remove excess stain. Gels can be scanned or dried using cellophane sheets to keep permanently.
- **5. Gel interpretation:** Gels are scored visually using a light box. Interpretation can also be conducted using our Kodak Imaging System.
- 6. Recipes:

#### 2CE extraction buffer

150ml 2-chloroethanol 350ml DI water 1 ml of 1% methyl green store at 4°C

#### Gel Buffer 1X

20 ml glacial acetic acid 1.0g glycine 1.45g ascorbic acid add DI water to 1 liter adjust pH to 3.1 if necessary

#### 10X Tank Buffer

160ml glacial acetic acid 16g glycine DI water to 4 liters

#### Gel recipe (2 gels)

68.75ml gel buffer 31.25ml 40% acrylamide 25ml 2% bis-acrylamide 300 ul of 0.1% FeSO4 use 110 ul of H2O2 to polymerize

#### G-250 stain solution

4 g G-250 200 ml 95% ethanol According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 051-0055. The time required to complete this information collection is estimated to average 1.5 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

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U.S. DEPARTMENT OF AGRICULTURE AGRICULTURAL MARKETING SERVICE SCIENCE AND TECHNOLOGY PLANT VARIETY PROTECTION OFFICE BELTSVILLE, MD 20705

Exhibit C

# OBJECTIVE DESCRIPTION OF VARIETY Oat (Avena spp.)

NAME OF APPLICANT (S)  NDSU Research Foundation	TEMPORARY OR EXPERIMENTAL DES	IGNATION	VARIETY NAME Souris						
ADDRESS (Street and No. or RD No., City, State, Zip Code, a c/o Executive Director NDSU Re PO Box 5002, Fargo, ND 58105-5	search Foundation		FOR OFFICIALUI PVPO NUMBER ##		0	8	0	0	0 (
Place the appropriate number that describes the (i.e. 0 8 9 or 0 9 ) when the number	varietal character of this variety in the	e boxes below. Plac	ce a zero in the fi	rst box					
1. SPECIES:  1 1 = Sativa 2 = Byzantina	3 = Other (Specify)								
2. GROWTH HABIT:  3    1 = Winter    2 = Semi-Winter  3    Juvenile Growth:   1 = Prostrate		Erect	\ 						-
	Pi								
4. PLANT HEIGHT: (From Soil Level to Top of B	Head)								
Same as Check * AC	Ronald lldeer								



<sup>\*</sup> Relative to a Commercial Variety Grown in the Same Trial

_				LUUUUU
. 5	2 1	Diameter: 1 = Fine (Kherson)  Hairiness at Upper Culm Nodes: 1 = Hairless	2 = Medium (Clintford) 3 = Co 2 = Hairy	parse (Nodaway 70)
	L	Mature Stem Color 1 = Yellow 2 = Reddish		
6.	LEAF: (Leval) 1 3 1 7 2	eaf Color: The Royal Horticultural Society's or any refriety.)  Carriage: 1 = Drooping (Random) 2 = Erect  Color: 1 = Yellow-Green 2 = Light  mm Width (First leaf below flag leaf)  Ligule: 1 = Absent 2 = Present	t (Walken)	= Blue-Green s 2 = Ciliate
7.	HEAD:			
	1 2 2 1 8	Panicle Size: 1 = Samll (Yancey) 2 = Me Panicle Width: 1 = Narrow (Gopher) 2 = Mid	st Node $2 = Second Node (False edium (Walken) 3 = Large (Markton) dbroad (Yancy) 3 = Broad (Nodawa nber of Branches 0 = 6 N (Yancey) 2 = Spreading (Oance)$	y 70) umber of Whorls of Branches ayuse) 3 = Drooping (Markton)
•	PACHIS:	1 = Recurved (Yancey) 2 = Erect (Walken)  Second Floret Rachilla Segment: 1 = Hairless 2 = Hairy	9 mm Second Floret I Rachilla Hairs: 1 = Short	Rachilla Segment Length 2 = Long
9.	3 2 2 1	Spikelet Separation by: 1 = Abscission 2 = 5	Semi-Abscission 3 = Fracture Heterofracture 3 = Basifracture	
10.	0 5	: (Glume Color: The Royal Horticultural Society's or described variety.)  mm Width  1 9 mm Length  0 8	any recognized color chart should be No. of Veins on Glumes	Color: 1 = White 2 = Yellow 3 = Red 4 = Striped
11.	LEMMA:	(Lemma Color: The Royal Horticultural Society's o	or any recognized color chart should be	used to determine the leaf color of the
•	1 4	described variety.) mm Length Hairiness of Dorsal Surface: 1 = Hairless 2 = H	1 Color: 1 = White 4 = Gray	2 = Yellow 3 = Red 5 = Black
40	ASSAUL /FI	of Floreth	•	
12.	AWN: (Fir	Occurrence: 1 = Absent (Walken) 2 = Infrequent (Yancey) 3 = Common (Chilocco) 4 = Frequent (Random)	Type: mm Awn Length	1 = Non-Twisted 2 = Twisted 3 = Twisted Geniculate

Florescence Under Ultraviolet Light: 1 = Florescent 2 = Non-Florescent  Basal Hair: 1 = Absent (Florida 501) 4 = Several to Numerous (Florilee) 2 = Absent to Few (Yancey) 5 = Numerous (Red Rustproof) 3 = Few to Several (Lee)  mm Basal Hair Length  gms per 1000 Seeds gms per 1000	13. SEED	:	C		•
4 = Several to Numerous (Florilee)  5 = Numerous (Red Rustproof)  1	1	Florescence Under Ultravi	olet Light: 1 = Florescent	2 = Non-Florescent	
2 9 8 gms per 1000 Seeds  2 6 0 % Groat Protein  14. INSECTS: (0 = Not Tested 1 = Susceptible 2 = Resistant)  O Cereal Leaf Beetle O Bluegrass Billbug O Grain Bug (C. Sayi) O Other (Specify)  15. DISEASE: (0 = Not Tested 1 = Susceptible 2 = Resistant) Halo Blight Powdery Mildew Septoria Leaf Blotch Soil-Borne Mosaic Virus Helminthosporium Leaf Blotch Specify Races Tested: Races Susceptible Races Resistant CR 230 Cr 254, NDCRC 05  TJS + NA67 NA27	. 1			2 = Absent to Few (Yancey) 5 = Numerous (Red Rustproof)	3 = Few to Several (Lee)
gms per 1000 Seeds  14. INSECTS: (0 = Not Tested 1 = Susceptible 2 = Resistant)  O Green Bug (Biotype)  O Green Bug (C. Sayi)  O Nematode (Type)  O Nematode (Type)  O Other (Specify)		mm Basal Hair Length			
### Second Protein  ### Se	2 9	gms per 1000 Seeds	3 0	mg Groat Weight (each)	
O Cereal Leaf Beetle O Bluegrass Billbug O Grain Bug (C. Sayi) O Nematode (Type) Other (Specify)  15. DISEASE: (0 = Not Tested 1 = Susceptible 2 = Resistant) Halo Blight Powdery Mildew Septoria Leaf Blotch Soll-Borne Mosaic Virus Helminthosporium Leaf Blotch Victoria Blight Other (Specify)  Specify Races Tested: Races Susceptible Races Resistant CR 230 CR 254, NDCRC 05  TJS + NA67 NA27	1 6		6 4	% Groat Oil	
Office Bug (Biotype)  Other (Specify)  15. DISEASE: (0 = Not Tested 1 = Susceptible 2 = Resistant)  Halo Blight Powdery Mildew Septoria Leaf Blotch Soil-Borne Mosaic Virus Other (Specify)  Helminthosporium Leaf Blotch Specify Races Tested: Races Susceptible Races Resistant CR 230 CR 254, NDCRC 05  TJS + NA67 NA27	14. INSEC	TS: (0 = Not Tested 1 = Susc	eptible 2 = Resistant)		
15. DISEASE: (0 = Not Tested 1 = Susceptible 2 = Resistant)  Halo Blight Powdery Mildew Septoria Leaf Blotch Soil-Borne Mosaic Virus  Helminthosporium Leaf Blotch Virus Victoria Blight Other (Specify)  Specify Races Tested: Races Susceptible Races Resistant  CR 230 CR 254, NDCRC 05  TJS + NA67 NA27  Covered Smut	0	Cereal Leaf Beetle	O Bluegrass Billbug O Grai	n Bug (C. Sayi) 0 Nematod	e (Type)
Halo Blight Powdery Mildew Septoria Leaf Blotch Soil-Borne Mosaic Virus Helminthosporium Leaf Blotch Specify Races Tested: Races Susceptible Races Resistant Crown Rust Stem Rust Covered Smut  Powdery Mildew Septoria Leaf Blotch Soil-Borne Mosaic Virus Other (Specify)  Other (Specify)  Crown Rust CR 230 CR 254, NDCRC 05  TJS + NA67 NA27	0	Green Bug (Biotype)		Other (Specify)	:
Helminthosporium Leaf Blotch  Specify Races Tested: Crown Rust Stem Rust  Covered Smut  Yellow Dwarf Virus Victoria Blight  Other (Specify)  Races Resistant  CR 230  CR 254, NDCRC 05  NA27  TJS + NA67  NA27	15. DISEA	SE: (0 = Not Tested 1 = Susce	eptible 2 = Resistant)	·	
Crown Rust   CR 230   CR 254, NDCRC 05		Halo Blight P	owdery Mildew Septoria	Leaf Blotch Soil-Borne Mo	saic Virus
Cr 230   CR 254, NDCRC 05			ellow Dwarf Virus Victoria B	light Other (Specify	)
Cr 230   CR 254, NDCRC 05	-				
Crown Rust  Stem Rust  Crown Rust  TJS + NA67  NA27		Specify Races Tested:		•	Races Resistant
O Covered Smut	<del></del>	Crown Rust	CR 230	CR 254, NDC	CRC 05
Covered Smut		Stem Rust	TJS + NA67	NA27	
Loose Smut	닉	Covered Smut			
	0	Loose Smut			

### 16. INDICATE THE VARIETY YOU BELIEVE MOST CLOSELY TO RESEMBLE THAT SUBMITTED:

CHARACTER	VARIETY	CHARACTER	VARIETY
Plant Tillering	HiFi	Leaf Color	HiFi
Leaf Size	HiFi	Leaf Carriage	HiFi
Seed Color	HiFi	Seed Shape	HiFi

COMMENTS:

Exhibit D, Table 1

North Dakota Oat Variety Trial 2001-2005 Summary.

Crain Viola										
		Grain Yield			Test Weight					
·	2005	2004-05	2003-05	2002-05	2001-05	2005	2004-05	2003-05	2002-05	2001-05
	10 Loc	2 yr	3 yr	4 yr	5 yr	10 Loc	2 yr	3 yr	4 yr	5 yr
Genotype	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
	Lb/Bushhel									
Assiniboia AC	115	115	127	118	121	33.6	35.9	36.1	35.6	35.2
Beach	116	116	133	122	125	37.9	39.2	39.1	38.5	38.1
Ebeltoft	114	114	134	123	125	34.4	36.0	35.9	35.3	34.9
HiFi	136	136	140	127	129	37.0	38.2	38.0	37.2	37.0
Hytest	93	93	108	99	101	39.3	40.6	40.7	40.1	39.8
Jerry	95	95	117	109	111	36.6	38.4	38.7	38.2	37.8
Killdeer	123	123	142	130	131	34.9	36.8	36.7	36.1	35.6
Maida	117	117				37.0	38.0			
Morton	124	124	131	120	124	37.3	38.6	38.5	38.0	37.8
Otana	87	87	117	109	110	32.1	35.0	35.1	34.8	34.3
Souris	130	130	141	130	132	37.4	38.4	38.2	37.6	37.3
Loc/yr	10	20	28	36	42	10	20	28	36	42

# Exhibit D, Table 2.

North Dakota Oat Variety Trial 2001-2005 Summary.

Grain Quality										
		2003-	2005		Fargo Gro	at Beta-C	Glucan	Fa	rgo Groa	at Oil
		Kern	<5/64"		2003	2005	2 yr			2 yr
Genotype	2003	2004	2005	3 yr	BG		2003,05	2003	2005	2003,05
	Proportion % %									
Assiniboia AC	0.07	0.043	0.130	0.056	2.8	5.1	4.0	9.5	9.2	9.4
Beach	0.11	0.075	0.132	0.089	4.9	5.7	5.3	10.1	9.8	10.0
HiFi	0.07	0.062	0.222	0.067	6.1	7.5	6.8	9.0	8.9	8.9
Hytest	0.09	0.078	0.117	0.084	5.2	6.2	5.7	7.7	7.5	7.6
Jerry	0.05	0.032	0.171	0.040	3.7	5.5	4.6	7.4	7.2	7.3
Killdeer	0.11	0.075	0.154	0.089	4.6	6.7	5.7	7.8	8.3	8.1
Maida	0.12	0.085	0.101	0.099		5.4			8.6	
Morton	0.20	0.171	0.128	0.183	4.5	5.0	4.8	7.4	7.0	7.2
Otana	0.63	0.592	0.264	0.610	5.4	6.1	5.7	7.6	8.6	8.1
Souris	0.19	0.160	0.193	0.172	4.9	5.5	5.2	7.3	6.9	7.1
Loc/yr	9	10	9	28	1	1	2	. 1	1	2,0

Exhibit D, Table 3. 2005-2007 Oat Variety Trial Head and Height Summary Over LocationsTrait Summary Over Locations

		Headin	g Date >	31-May			PI	ant Heig	ht	
Genotype	2005	2006	2007	2006-07	2005-07	2005	2006	2007	2006-07	2005-07
			days					cm		
AC Assiniboia	31.1	31.1	24.8	28.0	29.0	103	89	96	93	96
Beach	28.9	28.9	22.5	25.7	26.8	109	96	105	101	104
HiFi	28.3	28.3	23.5	25.9	26.7	106	91	98	94	98
Hytest	27.4	27.4	20.2	23.8	25.0	108	96	105	100	103
Jerry	26.9	26.9	20.4	23.6	24.7	105	90	100	95	98
Killdeer	28.0	28.0	21.9	24.9	25.9	. 96	81	88	84	88
Maida	29.0	29.0	22.2	25.6	26.7	106	91	.99	95	99
Morton	29.5	29.5	23.4	26.4	27.5	113	96	107	101.	105
Otana	30.3	30.3	24.4	27.3	28.3	108	96	99	97	101
Souris	28.9	28.9	22.8	25.8	26.9	99	82	93	88	92
Youngs	30.1	30.1	23.9	27.0	28.1	107	95	105	100	102
EXP MEAN	29.7	29.7	23.3	25.8	26.9	105	80	99.8	90.0	95.2
No. Locations	8 loc	8 loc	7 loc	15 loc	23 loc	8 loc	8 loc	6 loc	14 loc	22 loc

Exhibit D, Table 4

Seedling infection type after inoculation with crown rust composite NDCR05

Genotype	2005	2006	2007
		IT	
AC Assiniboia	4	4	4
Beach	4	4	4
HiFi	;	;	i
Hytest	4	4	4
Jerry	4	4	4
Killdeer	4	4	4
Maida	4	4	4
Morton	4	4	4
Otana	4	4	4
Souris	;	;	;
Youngs	4	4	4

Exhibit D, Table 5
Seedling infection type after inoculation with stem rust race NA67.

Genotype	2005	2006	2007
		IT	
AC Assiniboia	2	2	2
Beach	2	- 2	2
HiFi	2	2	2
Hytest	4	4	4
Jerry	2	2	2
Killdeer	2	2	2
Maida	<u> </u>	;	;
Morton	2	2	2
Otana	4	4	4
Souris	1	1	1
Youngs	4	4	4



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1. NAME OF APPLICANT(S)	2. TEMPORARY DESIGNATION	3. VARIETY NAME
`,	OR EXPERIMENTAL NUMBER	
NDSU Research Foundation	ND961161	Souris
4. ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP, and Country)	5. TELEPHONE (Include area code)	6, FAX (include area code)
,		
c/o Executive Director PO Box 5002	(701) 231-8931	(701) 231-6661
Fargo, ND 58102-5002	7. PVPO NUMBER	•
	#	200800063
8. Does the applicant own all rights to the variety? Mark an "X" in the	ne appropriate block. If no. please expla	in. YES NO
9. Is the applicant (individual or company) a U.S. national or a U.S. t	based company? If no, give name of co	puntry. TYES NO
o. to the applicant (individual of company) a c.c. handral of a c.c. i	based company? If no, give name of co	ountry.
10. Is the applicant the original owner? YES	NO If no, please answer one	of the following:
a. If the original rights to variety were owned by individual(s), is	(are) the original owner(s) a U.S. National NO If no, give name of count	
✓ YES	NO If no, give name of countr	
11. Additional explanation on ownership (Trace ownership from original contents)	inal breeder to current owner. Use the re	everse for extra space if needed):
See additional Exhibit E. Statement of the Basis of the Applican	at's ownership included in the application	a.
	•	
PLEASE NOTE:		
Plant variety protection can only be afforded to the owners (not licens	sees) who meet the following criteria:	
<ol> <li>If the rights to the variety are owned by the original breeder, that p national of a country which affords similar protection to nationals o</li> </ol>		
<ol><li>If the rights to the variety are owned by the company which employ nationals of a UPOV member country, or owned by nationals of a genus and species.</li></ol>	yed the original breeder(s), the company country which affords similar protection t	must be U.S. based, owned by o nationals of the U.S. for the same
3. If the applicant is an owner who is not the original owner, both the	original owner and the applicant must m	eet one of the above criteria.
The original breeder/owner may be the individual or company who di Act for definitions.	irected the final breeding. See Section 4	1(a)(2) of the Plant Variety Protection
According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, control number. The valid OMB control number for this information collection is 0581-0055, including the time for reviewing the instructions, searching existing data sources, gathering a	. The time required to complete this information collec	tion is estimated to average 0.1 hour per response,
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ST-470-E (04-03) designed by the Plant Variety Protection Office using Word 2000

19e. Exhibit E. Statement of the Basis of the Owner's Ownership

Dr. Michael S. McMullen, an employee of the North Dakota Agricultural Experiment Station and North Dakota State University, is a plant breeder who developed 'Souris' spring oat for which Plant Variety Protection is hereby sought. The employee by agreement and because of the condition of the use of facilities and funds of the North Dakota Agricultural Experiment Station and North Dakota State University has assigned all ownership rights to Souris oat to the North Dakota Agricultural Experiment Station and the North Dakota State University.

North Dakota State University on behalf of the North Dakota Agricultural Experiment Station has assigned all ownership to the NDSU Research Foundation. NDSU/RF is a nonprofit corporation set up to own and manage the intellectual property of North Dakota State University.

Form Approved OMB NO 0581-0055
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> U.S. DEPARTMENT OF AGRICULTURE **AGRICULTURAL MARKETING SERVICE** SCIENCE AND TECHNOLOGY PLANT VARIETY PROTECTION OFFICE BELTSVILLE, MD 20705

**EXHIBIT F** 

	DECLARATION REGARDING DEPOSIT	
NAME OF OWNER (S)	ADDRESS (Street and No. or RD No., City, State, and Zip Code and Country)	TEMPORARY OR EXPERIMENTAL DESIGNATION
NDSU Research Foundation	1735 NDSU Research Park Drive	ND961161
	Fargo, ND 58105	VARIETY NAME 'Souris'
NAME OF OWNER REPRESENTATIVE (S)	ADDRESS (Street and No. or RD No., City, State, and Zip Code and Country)	FOR OFFICIAL USE ONLY
Dale Zetocha	1735 NDSU Research Park Drive Fargo, ND 58105	200800063

I do hereby declare that during the life of the certificate a viable sample of propagating material of the subject variety will be deposited, and replenished as needed periodically, in a public repository in the United States in accordance with the regulations established by the Plant Variety Protection Office.

Michael McMullen, NDSU Breeder